THE PHARMACOKINETICS OF PENTOSAN POLYSULPHATE (PPS) AS A POTENTIAL PROPHYLACTIC AGAINST TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHY (TSE)

Introduction[1]

PPS is a polysulphonated polyglycoside (PG), a group of which have been investigated since 1982 as potential therapeutic agents against scrapie in mice and hamsters. It was found that when PGs were given as inocula to the periphery within a period of the disease inocula (generally between 2 months before to 4 weeks afterwards) then the apparent inoculum dose of the disease was decreased and, if the disease dose was low, no infection took place. The most effective PG in this respect was PPS.

PG's were found to replace heparan which binds to the normal prion protein (PrPc) during the production of the prion. Heparan is required for the stability of amyloids (prions are retained as amyloid fibrils) and their protection from cellular enzymes. It has been shown that for other amyloids heparan is needed for the formation of the amyloid fibrils, and if heparan is displaced from them *in vivo* experiments then the fibrils are destroyed. It is felt that prions are formed from PrPc by the interaction with fibrillar cystalloids of prion already present. When PPS or other PG's are present they prevent further prion formation taking place *in vivo* and *in vitro*. The continual breakdown of prions in the reticuloendothelial system (RES), presumably due to cellular turnover, permits the disease to be removed from the animal's body and no infection is found in animals that have been treated with PPS when tissues are inoculated into further animals. *In vitro* PPS is found to be taken up and concentrated inside the cells of the RES and this may explain why only 1 ng/ml is required in the culture fluid of infected mouse neuroblastoma cells for 50% inhibition of prion production and when the PPS was removed from this fluid prion production did not return. This remains to be explained in that the prion crystalloids must have remained within the infected cells but simply could no longer induce the alteration of PrPc to the prion form. It was as if the PPS 'capped' the growing crystalloid.

Both PrPc structure and that of heparan are well preserved through evolution. As such, the finding, that human PrPc also carried a binding site and that human PrP-amyloid also contained large amounts of heparan was not surprising. Scrapie PrP-amyloid also contains heparan and binds with Congo red on the same binding site.

The importance of the pharmacokinetics of PPS

The prevalence of nvCJD amongst the UK community is currently unknown and pre-clinical diagnostic tests are inadequate but, because calculations suggest that it would be transmitted from one human to another through blood transfusion and 2 million units of blood are used in the UK annually for separation or transfusion, a prophylactic measure would be of value.

When considering the potential of PPS as such a prophylactic agent, it is important to be sure that the drug is present in the similar sites and times within the body which has been shown to cause a preventative effect in rodents. Also, the use of PPS as an oral human drug would demand that adequate oral absorption and

effects similar to intravenous inoculum could be demonstrated. The current large amounts of data on the lack of oral toxicity of PPS is required when considering it as a prophylactic as the prevalence of nvCJD in the community is unknown and many people that are not at risk from the disease may be offered the drug.

Current information on the oral absorption of PG's in humans

The large molecular weight and ionically charged nature of PG's would suggest poor absorption across cellular membranes of the gut. There has been wide research into this in attempts to treat people with PG's for other purposes. Initial tests with heparan were surprising in that absorption rates of around 10% were found[2]. Much greater research was carried out into this in both animals[3-11] and in man[12-14]. The mechanism of absorption was never fully understood but was clear that it was fragments of the original heparin molecule that arrived in the plasma and this was followed by rapid uptake into vascular endothelium and reticuloendothelial system (RES) cells and so very low plasma levels were found. Inside the cells the heparin is broken down by endo-b-glucuronidase and then by lysosomal enzymes. As such, when small doses reached the plasma, through oral administration, minimal urinary excretion would be expected. The literature contains numerous reports of attempts to administer it orally but with little success in achieving clinically useful anticoagulation. Attempts have been made to improve enteral absorption in a number of ways: use of adjuvants such as EDTA[15], citric acid[16], DMSO[17,] administration in micelle forms or in liposomes[18], rectal administration with bile salts[19] or as a low molecular weight form[20].

Further PG's were found to be absorbed in the gut: dextran sulphate[21], chondroitin sulphate[14, 22, 23,] dermatan sulphate[24-26] and carageenans[27]. What could be seen was that highly sulphated PG's tend to be taken up into the plasma and cleared into RES tissues faster than less sulphated ones. Experimentation can show that they are bound more highly to heparan binding sites on cellular proteins. As they are taken up into RES cells and either destroyed (chondroitin), desulphated (PPS), or retained (dextran sulphate) it is extremely difficult to assess the uptake of PG's through their measurement by anticoagulation or through plasma concentrations. A more useful manner would be through an assessment of uptake into the RES using radioisotopes but little quantitative work has been done on this.

Much research has been attempted into the clearance of Pentosan from the plasma following intravenous or subcutaneous administration. Unlike heparin it is not wholly destroyed after uptake into RES or endothelial cells but is concentrated inside the cells themselves, gradually desulphonated and excreted in the urine over a relatively long period, showing a renal clearance over approximately a week[28-32].

Oral administration pharmacokinetics of PPS have been carried out by the manufacturers for the licensing of the drug in the USA as a treatment for interstitial cystitis[33-55]. Its oral absorption rate in healthy males was found to be 2%-4% but this is considered an underestimate as only plasma and urine levels were used as indicators of this. Its mode of action in this condition depends on the uptake into fibroblasts; the low levels found in the plasma suggest that, like heparin, it is taken up actively into cells and concentrated within them[36,37]. The many actions of PPS have also caused its oral use in other conditions: advanced cancer [38] (due to its

ability to inhibit the growth of vascular tubes), radiation-induced proctitis[39] (due to fibroblast inhibition), osteoarthritis[40-43] (due to its stimulation of chondroblasts and hyaluronic acid production) and prevention of the production of renal calculi[43-47] (due to its ability to inhibit crystal growth). In each of these there was clear indication that the drug was absorbed to some degree and had clinical effects.

Because of these previous findings, the comparative pharmacokinetics between the mouse and the human should be expected to be successful in showing that it is taken up into the RES in both, concentrated within the cells, and excreted over a longer period. As such a comparison in its effectiveness as a potential prophylactic against scrapie in mice and nvCJD in humans may be possible.

There is little data available on the long term organ distribution and pharmacokinetics of PPS in the mouse model. Some limited data is available on the blood clearance of labelled Pentosan derivative in the rabbit[49] and its metabolism in man [28].

Experimental Design

A derivative of Pentosan polysulphate containing tyrosyl residues can be synthesized[49,28]. Radioiodination of this derivative using ^{125}I, ^{131}I or ^{123}I may be achieved using conventional iodination methods such as chloramine T or Iodogen[50,51]. The characterization of the radioiodinated derivative would be determined by chromatographic techniques. The *in vitro* biological assessment of the radiolabelled compound would be carried out by measuring the anticoagulant activity using the activated partial thromboplastin time assay and Pentosan would be used as a control.

The *in vivo* biodistribution of the radiolabelled derivative would be investigated in normal small rodents: In order to obtain a complete picture of the fate of labelled Pentosan derivative; the uptake and elimination in all the major body organs, e.g. liver, spleen, lungs, kidneys, blood (including white blood cell types), muscle, gonads, brain and bone would be determined following intravenous injection at several time points.

The plasma and urine samples will be analysed using chromatographic techniques to investigate the presence of metabolic products.

The preferred route of dosage for therapeutic use would be by oral administration. The blood appearance data would be acquired following the oral administration of the labelled compound under various physiological conditions of the gastro intestinal tract and with varying dosage regimes. The biodistribution data would also be obtained as for the intravenous route. These measurements will provide a quantitative measure of bioavailability following the oral dose.
It has been suggested that liver and kidneys are the major organs for the desulphation of heparin *in vivo*[28]. Autoradiographic studies would be carried out in sections of these organs to obtain data of microdistribution of the radiolabelled Pentosan derivative within the cells of these organs.

The Future

It is hoped that we could develop from the animal work described above to obtain biodistribution data in humans with a view to developing a non-invasive diagnostic test using labelled Pentosan or similar agents to detect prion infection. The difficulty foreseen in humans is that it would be unethical to obtain biopsy samples for biodistribution and external imaging with radioactive materials will be limited by the radiation dosimetry. This will have to be estimated from the animal data above before an application can be made to the Administration of Radioactive Substances Advisory Committee to proceed to a normal volunteer study, which would be possible by substituting the ^{125}I or ^{131}I label for the ^{123}I label. Useful information may also be obtained from the relationship of the activity in the lymphocytes to the biodistribution in the reticuloendothelial system as a whole. If this is the case it may be possible to use blood samples and the ^{125}I label in normal human volunteers. This may also be an avenue to explore when looking for a diagnostic test of prion infection.

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